Supplemental Material

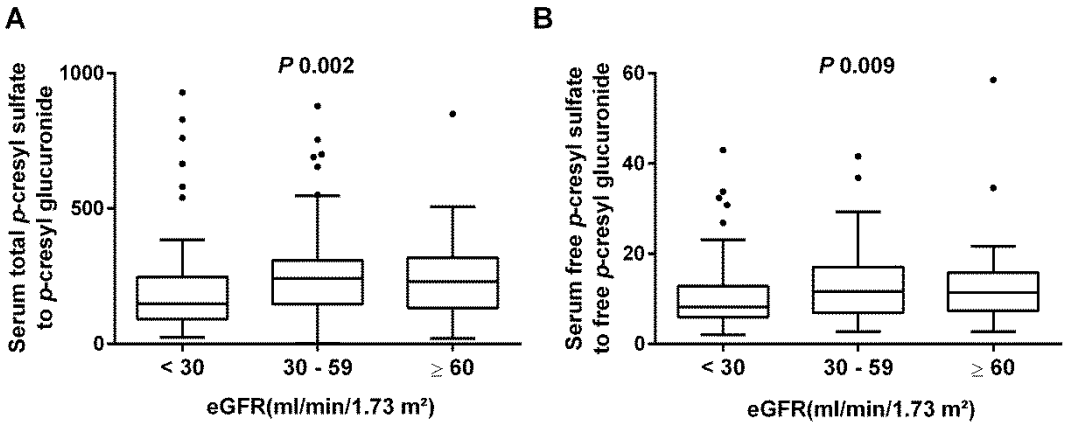
Detailed description of UPLC-MS/MS method

Serum levels of *p*-cresyl sulfate (PCS) and *p*-cresyl glucuronide (PCG) were quantified using a dedicated ultra-performance liquid chromatography – tandem mass spectrometry (UPLC–MS/MS) (Acquity – Xevo TQS, Waters, Zellik, Belgium) method. For sample preparation, 50 µl serum, 50 µl solution of MQ water/MeOH/0.01 N sodium hydroxide (% v/v 75/20/5), 20 µl of internal standard mixture (i.e., PCS-d7 and PCG-d4) and 150 µl acetonitrile were thoroughly mixed in 96-well Ostro plates (Waters, Zellik, Belgium). After separation by a positive pressure manifold, supernatants were collected in 2-ml collection plates. Subsequently, the organic phase was removed by a gentle stream of nitrogen for 30 minutes at 40 °C. After dilution with 1000 µl MQ water, 5 µl of the final solution was injected on the UPLC-MS/MS system. Chromatographic separation was performed on an Acquity CSHFluoroPhenyl column (Waters, Zellik, Belgium). Ionization of PCS, PCG and their corresponding isotopologues (internal standards) was achieved in negative mode. The following multiple reaction monitoring (MRM) transitions were used for quantification: *m/z* PCS 187 → 107, PCS-d7 194 → 114, PCG 283 → 113 and PCG-d4 287 → 113. Limits of detection (LOD) and limits of quantification (LOQ) were 0.03 µM and 0.286 µM, respectively, for PCS, and 0.01 µM and 0.035 µM for PCG. For analysis, solute levels below the LOQ were treated as the average value of the LOD and LOQ.

Detailed description of endpoint evaluation

Adjudication was by a single physician and checked by a second physician. Differences between primary and secondary adjudication were resolved by discussion. Physicians were blinded from biochemical results until adjudication was completed. For cardiovascular events, we considered a composite of death from cardiac causes, non-lethal myocardial infarction, myocardial ischemia, coronary intervention, ischemic stroke or new-onset peripheral vascular disease, whichever occurred first. Only one event per subject was included in the analysis. After review of available information from medical records, cause of death was classified as either cardiovascular, infectious, malignancy or other. Cardiovascular deaths included fatal myocardial infarction, sudden death and death due to congestive heart failure. Cases of unobserved sudden death were considered cardiovascular death only when other potential causes could be excluded. Otherwise, they were classified as other cause of death. Out-of-hospital deaths were coded after consultation of the general practitioner. Non-lethal cardiovascular events included myocardial infarction, diagnosed based on elevated levels of cardiac enzymes and/or typical electrocardiography changes, myocardial ischemia with typical electrocardiography changes without elevated cardiac enzymes, coronary intervention (thrombolysis, percutaneous coronary intervention or coronary artery bypass grafting) and ventricular arrhythmia. Ischemic stroke was defined as a neurologic deficit lasting more than 24 hours. Hemorrhagic stroke was excluded from the primary endpoint. Peripheral vascular disease included new-onset ischemic pain in the lower limbs, with abnormal ankle brachial pressure index or radiologic evidence of peripheral vascular disease, new-onset ischemic necrotic lesions or surgical arterial intervention.

Supplemental Figure 1 – Relationship between proportion of serum *p*-cresyl sulfate to *p*-cresyl glucuronide and renal function (Kruskal-Wallis test)



Supplemental Table 1 – Cox proportional hazards analysis of overall mortality and cardiovascular disease for serum total *p*-cresol and proportion of serum total *p*-cresyl glucuronide to *p*-cresol

	Variable	Hazard ratio per SD higher (95 % confidence interval)	*P*
MORTALITY	Model 1: *p*-cresol (Ln)	1.88 (1.37 – 2.59)	< 0.001
	p-cresyl glucuronide to *p*-cresol (Ln)	1.72 (1.30 – 2.29)	< 0.001
	Model 2: *p*-cresol (Ln)	1.57 (1.09 – 2.27)	0.02
	p-cresyl glucuronide to *p*-cresol (Ln)	1.56 (1.12 – 2.17)	0.008
CARDIOVASCULAR DISEASE	Model 1: *p*-cresol (Ln)	2.09 (1.60 – 2.73)	< 0.001
	p-cresyl glucuronide to *p*-cresol (Ln)	1.86 (1.43 – 2.42)	< 0.001
	Model 2: *p*-cresol (Ln)	1.67 (1.27 – 2.21)	< 0.001
	p-cresyl glucuronide to *p*-cresol (Ln)	1.81 (1.38 – 2.37)	< 0.001

Model 1 included serum total *p*-cresol and proportion of serum total *p*-cresyl glucuronide to *p*-cresol.
Model 2 included additional adjustment for eGFR (Ln), age, gender, systolic blood pressure, current smoker, diabetes mellitus, cholesterol, calcium, phosphate, parathyroid hormone (Ln), c-reactive protein (Ln), albumin.
Serum total *p*-cresol is the sum of serum total *p*-cresyl sulfate and *p*-cresyl glucuronide.
eGFR, estimated glomerular filtration rate

Supplemental Table 2 – Cox proportional hazards analysis of overall mortality and cardiovascular disease for serum total *p*-cresyl glucuronide

	Variable	Hazard ratio per SD higher (95 % confidence interval)	*P*
MORTALITY	1. Unadjusted	2.02 (1.49 – 2.73)	< 0.001
	2. Adjustment for eGFR (Ln), age, gender, systolic blood pressure, current smoker, diabetes mellitus, cholesterol, calcium, phosphate, parathyroid hormone (Ln), c-reactive protein (Ln), albumin	1.70 (1.21 – 2.38)	0.002
CARDIOVASCULAR DISEASE	1. Unadjusted	2.26 (1.76 – 2.90)	< 0.001
	2. Adjustment for eGFR (Ln), age, gender, systolic blood pressure, current smoker, diabetes mellitus, cholesterol, calcium, phosphate, parathyroid hormone (Ln), c-reactive protein (Ln), albumin	1.91 (1.46 – 2.50)	< 0.001

eGFR, estimated glomerular filtration rate